

REMARKS

The applicant has elected for further prosecution in the present application the invention of Group I, including claims 38-64. The applicant does not waive any right to have the invention of Group II, including claim 65 examined as originally filed without reduction in breadth in a subsequent continuing application.

The applicant has further amended the application to add limitations which enumerate all the specific components in the composition as required by the office in the detailed action.

The office indicates that claim 38 is generic. The applicant elects the following disclosed species for prosecution if no generic claim is finally held allowable as follows:

~~38/39 (equine sperm cells)/47/49 (200 mM Tris[hydroxymethyl]aminomethane/65mM
citric acid monohydrate)/50/51/52 (20% egg yolk)/53/54 (56mM fructose)/57/59 (6%
glycerol)/64/66/67~~

(Tris) ✓

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See
pg. 456
of Salisbury

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

38 (once amended). A method of freezing sex-selected sperm cells, comprising:

- (a) obtaining sperm cells from a male of a species of mammal;
- (b) sorting said sperm cells based upon sex-type;
- (c) cooling sex-selected sperm cells;
- (d) isolating a portion of said sex-selected sperm cells;
- (e) suspending said portion of said sex-selected sperm cells in an extender; [and]
- (f) freezing said sex-selected sperm cells in said extender; and
- (g) thawing said sex-selected sperm cells to provide fertile sex-selected sperm cells
[upon thawing].

39 (once amended). The method of freezing sex-selected sperm cells as described in claim 38, wherein said sperm cells from said species of said male mammal are selected from the group consisting of [comprise] bovine sperm cells and equine sperm cells.

40. The method of freezing sperm cells as described in claim 39, wherein said step of isolating a portion of said sex-selected sperm cells comprises isolating a number of bovine sperm cells between about 300,000 and about 3,000,000.

41. The method of freezing sperm cells as described in claim 39, wherein said step of isolating a portion of said sex-selected sperm cells comprises isolating a number of bovine sperm cells of no more than about 1,000,000.

42. The method of freezing sex-selected sperm cells as described in claim 38, wherein said sperm cells from said species of said male mammal comprise equine sperm cells.

43. The method of freezing sex-selected sperm cells as described in claim 42, wherein said step of isolating a portion of said sex-selected sperm cells comprises isolating a number of equine sperm cells between about 1,000,000 million and about 25,000,000.

44. The method of freezing sex-selected sperm cells as described in claim 42, wherein said step of isolating a portion of said sex-selected sperm cells comprises isolating a number of equine sperm cells of no more than about 5,000,000.

45. The method of freezing sex-selected sperm cells as described in claim 38, wherein said step of cooling sex-selected sperm cells comprises reducing the temperature of said sex-selected sperm cells to about 5°Celsius.

46. The method of freezing sex-selected sperm cells as described in claim 45, wherein said step of reducing the temperature of said sex-selected sperm cells comprises reducing the temperature of said sex-selected sperm cells over a period of about 60 minutes to about 240 minutes.

47. The method of freezing sex-selected sperm cells as described in claim 38, wherein said extender further comprises a component which maintains osmolality and buffers pH.

48. The method of freezing sex-selected sperm cells as described in claim 47, wherein said component which maintains osmolality and buffers pH is selected from the group consisting of a buffer comprising a salt, a buffer containing a carbohydrate, and any combination thereof.

49 (once amended). The method of freezing sex-selected sperm cells as described in claim 47, wherein said component which maintains osmolality and buffers pH is selected from the group consisting of sodium citrate, Tris[hydroxymethyl]aminomethane, 200mM Tris[hydroxymethyl]aminomethane, 175 mM to 225mM Tris[hydroxymethyl]aminomethane, 200 mM Tris[hydroxymethyl]aminomethane/65mM citric acid monohydrate, 175 mM to 225mM Tris[hydroxymethyl]aminomethane/50mM to 70mM citric acid monohydrate, N-Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid, 200 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/65 mM citric acid monohydrate, 175 mM to 225 mM Tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid/50mM to 70 mM citric acid monohydrate, monosodium glutamate,

milk, HEPES buffered medium, and any combination thereof.

50. The method of freezing sex-selected sperm cells as described in claim 47, 48, or 49, wherein said extender has a pH in the range of about 6.5 to about 7.5.

51 (once amended). The method of freezing sex-selected sperm cells as described in claim [47] 50, wherein said extender further comprises a cold shock protectant.

52 (once amended). The method of freezing sex-selected sperm cells as described in claim 51, wherein said cold shock protectant is selected from the group consisting of egg yolk, 20% egg yolk, 15% to 25% egg yolk, an egg yolk extract, milk, a milk extract, casein, albumin, lecithin, and any combination thereof.

53. The method of freezing sex-selected sperm cells as described in claim 51, wherein said extender further comprises an energy source.

54 (once amended). The method of freezing sex-selected sperm cells as described in claim 53, wherein said energy source is selected from the group consisting of a saccharide, glucose, fructose, 56 mM fructose, 45mM to 60mM fructose, mannose, and any combination thereof.

55. The method of freezing sex-selected sperm cells as described in claim 53, wherein said extender further comprises an antibiotic.

56. The method of freezing sex-selected sperm cells as described in claim 55, wherein said antibiotic is selected from the group consisting of tylosin, gentamicin, lincomycin, linco-spectin, spectinomycin, penicillin, streptomycin, and any combination thereof.

57. The method of freezing sex-selected sperm cells as described in claim 47, 51, 53, or 55, wherein said extender further comprises a cryoprotectant.

58. The method of freezing sex-selected sperm cells as described in claim 57, wherein said

cryoprotectant is selected from the group consisting of disaccharides, trisaccharides, and any combination thereof.

59 (once amended). The method of freezing sex-selected sperm cells as described in claim 57, wherein said cryoprotectant is selected from the group consisting of glycerol, 6% glycerol, between 5% to 7% glycerol, dimethyl sulfoxide, ethylene glycol, propylene glycol, and any combination thereof.

60. The method of freezing sex-selected sperm cells as described in claim 38, wherein the extender in which said portion of said sex-selected sperm cells is suspended comprises glycerol, sodium citrate, Tris[hydroxymethyl]aminomethane, egg yolk, fructose, and one or more antibiotics.

61. The method of freezing sex-selected sperm cells as described in claim 38, wherein the extender in which said portion of said sex-selected sperm cells is suspended comprises glycerol, sodium citrate, egg yolk, and one or more antibiotics.

62. The method of freezing sex-selected sperm cells as described in claim 38, wherein the extender in which said portion of said sex-selected sperm cells is suspended comprises glycerol, egg yolk, milk, fructose, and one or more antibiotics.

63. The method of freezing sex-selected sperm cells as described in claim 38, further comprising the step of equilibrating said portion of said sex-selected sperm cells suspended in said extender prior to freezing over a period of about 1 hour to about 18 hours.

64. The method of freezing sex-selected sperm cells as described in claim [38] 59, further comprising the step of equilibrating said portion of said sex-selected sperm cells suspended in said extender prior to freezing over a period of not greater than 6 hours.

65. A frozen sex-selected sperm sample in accordance with the method of claim 38.


(newly added) -- 66. The method of freezing sex-selected sperm cells as described in claim 39, wherein said step of suspending said portion of said sperm cells in said extender results in a final concentration of said sex-selected sperm cells of greater than 15 million per milliliter of extender.

67. The method of freezing sperm cells as described in claim 66, wherein said step of freezing said sex-selected sperm cells in said extender comprises freezing a number of bovine sperm cells between about 300,000 and about 5,000,000.--

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Respectfully Submitted,
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